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#### (57) Abstract

The present invention is based on the finding that nucleic acids containing at least one unmethylated cytosine-guanine (CpG) dinucleotide affect immune responses in a subject. These nucleic acids containing at least one unmethylated cytosine-guanine (CpG) dinucleotide can be used to induce an immune response in a subject. The method includes administering to the subject a therapeutically effective amount of nucleic acid encoding an antigenic polypeptide, and a therapeutically effective amount of an oligonucleotide containing at least one unmethylated CpG dinucleotide. The invention also provides a method for treating a subject having or at risk of having viral-mediated disorder, comprising administering to the subject a therapeutically effective amount of a nucleic acid encoding an antigenic polypeptide and an effective amount of an oligonucleotide containing at least one unmethylated CpG dinucleotide.

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# USE OF NUCLEIC ACIDS CONTAINING UNMETHYLATED CpG DINUCLEOTIDE AS AN ADJUVANT

## FIELD OF THE INVENTION

This invention relates to generally to adjuvants, and specifically to the use of oligonucleotides having at least one unmethylated CpG dinucleotide (CpG ODN) as an adjuvant.

# BACKGROUND OF THE INVENTION

Bacterial DNA, but not vertebrate DNA, has direct immunostimulatory effects on peripheral blood mononuclear cells (PBMC) in vitro (Krieg et al., 1995). This lymphocyte activation is due to unmethylated CpG dinucleotides, which are present at the expected frequency in bacterial DNA (1/16), but are under-represented (CpG suppression, 1/50 to 1/60) and methylated in vertebrate DNA. Activation may also be triggered by addition of synthetic oligodeoxynucleotides (ODN) that contain an unmethylated CpG dinucleotide in a particular sequence context. It appears likely that the rapid immune activation in response to CpG DNA may have evolved as one component of the innate immune defense mechanisms that recognize structural patterns specific to microbial molecules.

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CpG DNA induces proliferation of almost all (>95%) B cells and increases immunoglobulin (Ig) secretion. This B cell activation by CpG DNA is T cell independent and antigen non-specific. However, B cell activation by low concentrations of CpG DNA has strong synergy with signals delivered through the B cell antigen receptor for both B cell proliferation and Ig secretion (Krieg et al., 1995). This strong synergy between the B cell signaling pathways triggered through the B cell antigen receptor and by CpG DNA promotes antigen specific immune responses. In addition to its direct effects on B cells,

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CpG DNA also directly activates monocytes, macrophages, and dendritic cells to secrete a variety of cytokines, including high levels of IL-12 (Klinman et al., 1996; Halpern et al., 1996; Cowdery et al., 1996). These cytokines stimulate natural killer (NK) cells to secrete g-interferon (IFN-g) and have increased lytic activity (Klinman et al., 1996, supra; Cowdery et al., 1996, supra; Yamamoto et al., 1992; Ballas et al., 1996). Overall, CpG DNA induces a Th1 like pattern of cytokine production dominated by IL-12 and IFN-g with little secretion of Th2 cytokines (Klinman et al., 1996). The strong direct effects (T cell independent) of CpG DNA on B cells, as well as the induction of cytokines which could have indirect effects on B-cells via T-help pathways, suggests utility of CpG DNA in the form of ODN as a vaccine adjuvant.

A DNA vaccine induces immune responses against an antigenic protein expressed in vivo from an introduced gene. The DNA vaccine is most often in the form of a plasmid DNA expression vector produced in bacteria and then purified and delivered to muscle or skin (see Vogel and Sarver, 1995; Brazolot Millan and Davis, 1997; Donnelly et al., 1996). DNA vaccines have been demonstrated to show efficacy against numerous viral, bacterial and parasitic diseases in animal models. Almost all studies show induction of very strong and long-lasting humoral and cell-mediated immune responses, and protection against live pathogen challenge (where it could be evaluated). The efficacy of DNA vaccines is attributed, at least in part, to the continuous in vivo synthesis of antigen that leads to efficient antigen presentation. In particular, endogenously-synthesized antigen is presented by class I MHC, leading to induction of CD8+ cytotoxic T lymphocytes (CTL). In contrast, most whole killed and subunit vaccines, where antigen is processed solely in the exogenous form, often fail to induce CTL. More recently however, it has been shown that the presence of unmethylated CpG motifs in the DNA vaccines is essential for the induction of immune responses against the antigen (Sato et al., 1996).

Hepatitis B virus (HBV) poses a serious world-wide health problem. The current HBV vaccines are subunit vaccines containing particles of HBV envelope protein(s) which

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include several B and T cell epitopes known collectively as HBV surface antigen (HBsAg). The HBsAg particles may be purified from the plasma of chronically infected individuals or more commonly are produced as recombinant proteins. These vaccines induce antibodies against HBsAg (anti-HBs), which confer protection if present in titers 10 milli-International Units per milliliter (mIU/ml) (Ellis, 1993). While the subunit vaccines are safe and generally efficacious, they fail to meet all current vaccination needs. For example, early vaccination of infants born to chronically infected mothers, as well as others in endemic areas, drastically reduces the rate of infection, but a significant proportion of these babies will still become chronically infected themselves. This could possibly be reduced if high titers of anti-HBs antibodies could be induced earlier and if there were HBV-specific CTL. In addition, there are certain individuals who fail to respond (non-responders) or do not attain protective levels of immunity (hypo-responders). Finally, there is an urgent need for an effective treatment for the estimated 350 million chronic carriers of HBV and a therapeutic vaccine could meet this need.

SUMMARY OF THE INVENTION

The present invention is based on the finding that nucleic acids containing at least one unmethylated cytosine-guanine (CpG) dinucleotide affect the immune response in a subject by activating natural killer cells (NK) or redirecting a subject's immune response from a Th2 to a Th1 response by inducing monocytic and other cells to produce Th1 cytokines. These nucleic acids containing at least one unmethylated CpG can be used as an adjuvant, specifically to induce an immune response against an antigenic protein.

In one embodiment, the invention provides a method of inducing an immune response in a subject by administering to the subject a therapeutically effective amount of a nucleic acid encoding an antigenic protein and a therapeutically effective amount of an oligonucleotide containing at least one unmethylated CpG dinucleotide.

In another embodiment, the invention provides a method for treating a subject having or at risk of having a virally mediated disorder by administering to the subject a

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therapeutically effective amount of a nucleic acid encoding an antigenic protein and an effective amount of an oligonucleotide containing at least one unmethylated CpG dinucleotide.

In further embodiment, the invention provides a method for treating a subject having or at risk of having a chronic viral infection by administering to the subject an effective amount of an antigenic polypeptide and an effective amount of an oligonucleotide containing at least one unmethylated CpG dinucleotide.

In another embodiment, a pharmaceutical composition containing an immumostimulatory CpG oligonucleotide and a nucleic acid encoding an antigenic protein in a pharmaceutically acceptable carrier is provided.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph illustrating humoral responses in BALB/c mice immunized with 1 g recombinant HBsAg protein alone, adsorbed onto alum (25 mg Al³+/mg HBsAg), with 100 g of immunostimulatory CpG ODN, or with both alum and CpG ODN. Each point represents the group mean (n=10) for titers of anti-HBs (total IgG) as determined in triplicate by end-point dilution ELISA assay. End-point titers were defined as the highest plasma dilution that resulted in an absorbance value (OD 450) two times greater than that of control non-immune plasma with a cut-off value of 0.05. The upper graph shows results on a linear scale and the lower graph shows results on a logarithmic scale (log<sub>10</sub>).

FIG. 2 is a graph illustrating humoral responses in BALB/c mice immunized with 1 g recombinant HBsAg protein with alum and with 0, 10, 100 or 500 g of CpG ODN added. Each point represents the group mean (n=10) for anti-HBs titers (total IgG) as determined by end-point dilution ELISA assay.